A Phase I Dose-finding Study of Combined Treatment with an Antisense Bcl-2 Oligonucleotide (Genasense) and Mitoxantrone in Patients with Metastatic Hormone-refractory Prostate Cancer[1]

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ABSTRACT

Purpose: **Bcl-2 is a negative prognostic indicator in prostate cancer, implicated in the development of androgen independence and treatment resistance, and is overexpressed in hormone-refractory prostate cancer (HRPC). Genasense is a phosphorothioate antisense oligonucleotide complementary to the *bcl-2* mRNA open reading frame that in preclinical studies has shown significant activity in inhibiting expression of Bcl-2, delaying androgen independence, and improving chemosensitivity in prostate and other cancer models. In this dose escalation study, we evaluated the combination of Genasense and mitoxantrone, a standard chemotherapy for patients with HRPC.**

Design: **Twenty-six patients with HRPC were treated at seven dose levels receiving Genasense at a dose ranging from 0.6 to 5.0 mg/kg/day and mitoxantrone from 4 mg/m^2 to 12 mg/m^2. Genasense was administered as a 14-day i.v. continuous infusion every 28 days with mitoxantrone given as an i.v. bolus on day 8.**

Results: **No dose-limiting toxicities were observed. Hematological toxicities were transient and included neutropenia, thrombocytopenia, and lymphopenia. Nonhematological toxicities included fatigue, fever, nausea, arthralgias, myalgias, and transient elevations in serum creatinine, none of which were severe. Two patients had >50% reductions in prostate-specific antigen. One patient, who received six cycles of Genasense at 1.2 mg/kg/day and a low dose (4 mg/m^2) of mitoxantrone, also had symptomatic improvement in bone pain. Peripheral blood lymphocyte Bcl-2 protein expression decreased in five of five patients given Genasense at 5mg/kg/day (mean change from baseline, −12.8%; SD, 16.4%) as assessed by flow cytometry. Serum concentrations of Genasense given at doses of 3 mg/kg/day and greater, exceeded 1 μg/ml.**

Conclusions: **Genasense and mitoxantrone are well tolerated in combination, and mitoxantrone can be delivered at a standard dose with biologically active doses of Genasense without significant additional toxicity. This observation allays concerns about trials that combine Genasense with full doses of other cytotoxic agents seeking greater evidence of activity.**

INTRODUCTION

Prostate cancer is the most common cancer diagnosis and the second most common cause of cancer death in men in North America (1). Initially, prostate cancer responds very well to castration therapy; however, this response is usually brief, lasting ~18 months in the metastatic setting (2). A rising PSA[3], and recurrence of symptoms herald the development of androgen independence. At this point, standard treatment options are limited, consisting of palliative radiotherapy or chemotherapy (3). Mitoxantrone has been approved by the United States Food and Drug Administration (FDA) for use in HRPC based on two randomized controlled trials demonstrating an improved palliative response rate as compared with corticosteroids alone (4, 5). The objective and PSA response rates with mitoxantrone were low, and there was no survival benefit seen with chemotherapy (4, 5), emphasizing the need for more effective treatment for this disease.

The *bcl-2* gene is the prototype of a novel class of oncogenes that contributes to neoplastic progression by enhancing tumor cell survival through the inhibition of apoptosis (6). Bcl-2 belongs to a growing family of apoptosis-regulatory gene products that act as either death antagonists or death agonists. The selective and competitive dimerization between pairs of these antagonists and agonists determines how a cell responds to an apoptotic signal (7) such as with chemotherapy. Furthermore,

Received 7/6/01; revised 9/18/01; accepted 9/19/01.
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[3] The abbreviations used are: PSA, prostate-specific antigen; HRPC, hormone-refractory prostate cancer; ECOG, Eastern Cooperative Oncology Group; MTD, maximum tolerated dose; CIVI, continuous i.v. infusion; DLT, dose-limiting toxicity; AUC, area under the curve.

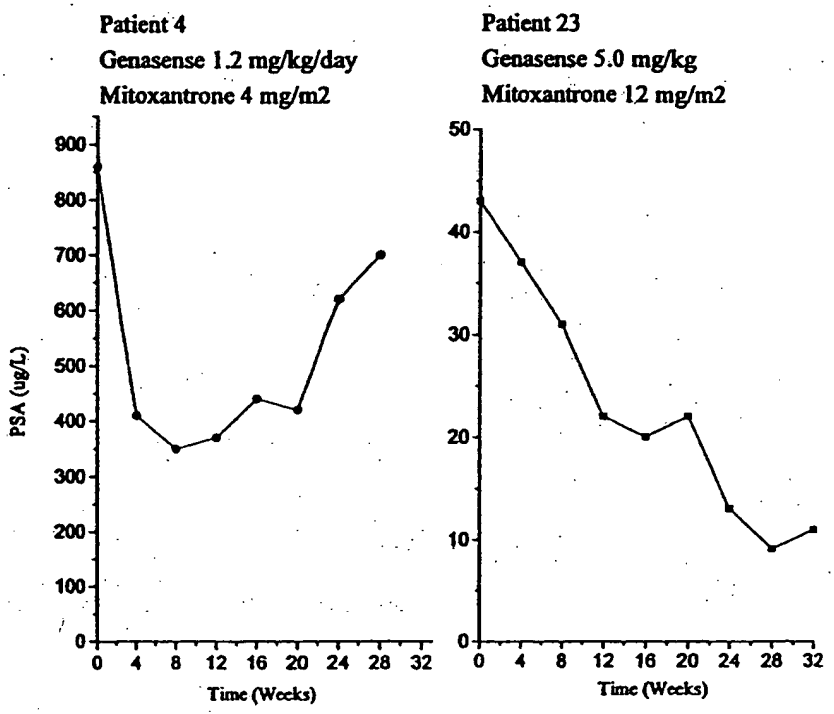

Fig. 1 PSA responses.

μg/ml. Mitoxantrone pharmacokinetic parameters at dose levels 6 and 7 are presented in Table 5. Mean *AUC* at dose levels 6 and 7 were 328.9 ± 52.6 ng·h/ml and 579.6 ± 172.7 ng·h/ml, respectively ($P = 0.008$). At dose levels 6 and 7, mean C_p were 77.8 ± 11.0 liters/h and 38.4 ± 11.51 liters/h, respectively ($P = 0.0003$).

DISCUSSION

This is the first study evaluating the use of Genasense, an antisense oligonucleotide complimentary to *bcl-2* mRNA, in combination with chemotherapy in patients with metastatic HRPC. Our results demonstrate that Genasense is well tolerated at doses of up to 5 mg/kg/day by CIVI for 14 days in combination with mitoxantrone chemotherapy at a standard dose of 12 mg/m^2 in this group of patients. No DLTs were seen at these doses, and patients on this trial were able to tolerate plasma concentrations of Genasense exceeding those achieved at MTD in a previously reported Phase I study (22). Furthermore, standard doses of mitoxantrone could be given to patients in whom Genasense plasma concentrations were above the biologically targeted concentration of 1 μg/ml (22, 26) with no significant excess toxicity being observed over and above what would be expected from mitoxantrone alone except for lymphopenia. There was no evidence to suggest cumulative toxicity with several patients receiving more than three cycles of therapy. Other trials evaluating combined therapy of Genasense with a cytotoxic agent have also reported good tolerability and MTDs that exceeded those in the initially reported Phase I trial of single-agent Genasense (27, 28).

Lymphopenia has been noted in other trials with Genasense (22, 27). This is consistent with an antisense effect, because mature lymphocytes seem to be dependent on Bcl-2 for survival (29). As in previous studies, the lymphopenia observed here was not of clinical significance with no increased occurrence of opportunistic infections. Significant thrombocytopenia, which was a DLT observed in a previous Phase I trial of Genasense as a single agent (22), was not seen in this study. Most toxicities that have been observed in other studies using antisense oligonucleotides have been non-sequence specific and have been attributed to the phosphorothioate backbone of these molecules (22, 27, 28, 30–32). Non-sequence specific toxicities have included fatigue and fever, which occurred in this study. Other reported toxicities with phosphorothioate oligonucleotides, such as elevations in transaminases, hyperglycemia, and alterations in coagulation parameters, were not observed in the present study.

We attempted to assess the biological effects of Genasense on its molecular target, Bcl-2. Ideally, an evaluation of a target effect should be made from neoplastic tissue; however with prostate cancer, patients present predominantly with bone-only disease and rarely present with nodal or visceral metastases, which would be easily amenable to repeated biopsy. Therefore, in this trial, we evaluated Bcl-2 expression in PBLs before and during the Genasense infusion, using a quantitative flow cytometric method as a possible surrogate tissue marker of biological activity. From our results, although minor changes did occur in the Bcl-2 expression that appeared dose related, an antisense effect could not be reliably described as being observed in the patient lymphocyte samples. Other investigators have recently found that Western analysis may be more sensitive in detecting decreases in PBL Bcl-2 expression than flow cytometry techniques (33). Further validation of tests for markers of target effect, accounting for normal baseline variation and indicating what constitutes a biologically meaningful decrease, and whether a surrogate tissue marker consistently reflects target tissue effect based on preclinical models, is required for future studies before they can be used as evidence of biological activity.

It can be speculated that a more pronounced target down-

Table 5 Plasma pharmacokinetic parameters of mitoxantrone following Genasense

Dose level	No. of patients	Genasense		Mitoxantrone							
		Dose (mg/kg/day)	C_{ss} (μg/ml)	Dose (mg/m^2)	$[AUC]_{0-\infty}$ (ng·h/ml)	C_p (ng/ml)	$t_{1/2\alpha}$ (h)	$t_{1/2\beta}$ (h)	V_c (liters)	V_{ss} (liters)	Cl (liters/h)
6	4	3.0	2.82 ± 0.66	12.0	328.9 ± 52.6	816.1 ± 202.2	0.21 ± 0.02	3.83 ± 3.1	32.8 ± 8.9	173.3 ± 198.4	77.8 ± 11.0
7	6	5.0	4.29 ± 0.52	12.0	579.6 ± 172.7	1473.4 ± 886.5	0.19 ± 0.06	5.48 ± 2.7	19.3 ± 10.2	144.9 ± 103.1	38.4 ± 11.51
P			0.0001		0.008	ns[a]	ns	ns	0.02	ns	0.0003

[a] $[AUC]_{0-\infty}$, area under the concentration-time curve; C_p, peak plasma concentration; C_{ss}, concentration in plasma at steady state; $t_{1/2\alpha}$, distribution half-life; $t_{1/2\beta}$, elimination half-life; V_c, central compartment volume of distribution; V_{ss}, volume of distribution at steady state; Cl, plasma clearance; ns, not significant.

low objective activity used for primarily palliative purposes (4, 5). Another possible explanation for the lack of a more pronounced clinical effect is that, for the reasons presented above, we did not sample patient tumors to assess for baseline overexpression of Bcl-2, and it is conceivable that only cancers that overexpress Bcl-2 are amenable to chemosensitization with Genasense. *In vitro* data however, suggests that this might not be the case and that an antisense effect can still be seen in cells that have either higher or lower expression of Bcl-2 (36). The median overall survival of the group was 18.7 months, which is significantly longer than the survival for patients with metastatic HRPC reported from randomized trials (4, 5). This certainly reflects patient selection, emphasizing that caution must be used when extrapolating survival data from early-phase clinical trials.

Recent published studies report that antimicrotubule regimens, most notably docetaxel, may have significantly more activity in prostate cancer, with response rates in the range of 30% for objective disease and 50% for PSA response in Phase II trials (37). A mechanism of action of antimicrotubule agents may be through the phosphorylation and, hence, the inactivation of Bcl-2 (38), and, therefore, the combination of Genasense with an antimicrotubule agent may be a more promising regimen for synergistic activity, as demonstrated in prostate xenograft studies (20, 21). Clinical trials that use this combination in HRPC are currently underway at our center and elsewhere.

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ALLOWED CLAIMS

11. The method of claim 19, wherein the cell is a cancer cell.

12. The method of claim 11, wherein said cancer cell is a follicular lymphoma cell.

13. The method of claim 19, wherein said first polynucleotide is an oligonucleotide having a length of between about 8 and about 50 bases.

14. The method of claim 19, comprising a liposome formed from the phospholipid.

15. The method of claim 14, wherein the liposome encapsulates the first polynucleotide.

18. The method of claim 19, wherein said composition is delivered to said human in a volume of 0.50-10.0 ml per dose.

19. A method of inhibiting proliferation of a Bcl-2-associated disease cell comprising obtaining a polynucleotide that hybridizes to Bcl-2 mRNA under intracellular conditions, mixing the first polynucleotide with a neutral phospholipid to form a composition comprising a polynucleotide/phospholipid association, and administering said composition to a human having a Bcl-2-associated disease to inhibit the proliferation of said disease cells, wherein said disease cells have a t(14;18) translocation, wherein said composition is delivered to said human in an amount of from about 5 to about 30 mg polynucleotide per m^2.

20. The method of claim 19, wherein said composition is administered three times per week for eight weeks.

22. The method of claim 29, wherein the cell is a cancer cell.

23. The method of claim 22, wherein said cancer cell is a follicular lymphoma cell.

24. The method of claim 29, comprising a liposome formed from the phospholipid.

25. The method of claim 24, wherein the liposome encapsulates the polynucleotide.

28. The method of claim 29, wherein said composition is delivered to said human in a volume of 0.50-10.0 ml per dose.

29. A method of inhibiting proliferation of a Bcl-2-associated disease cell having a t(14;18) translocation comprising:

(a) obtaining an oligonucleotide of from about 8 to about 50 bases that hybridizes to a Bcl-2-encoding polynucleotide under intracellular conditions;

(b) mixing the oligonucleotide with a neutral phospholipid to form a composition comprising a neutral oligonucleotide/phospholipid association; and

(c) administering said composition to said Bcl-2-associated disease cell to inhibit the proliferation of said disease cell,

wherein said cell is in a human, and wherein said composition is delivered to said human in an amount of from about 5 to about 30 mg polynucleotide per m^2.

30. The method of claim 29, wherein said composition is administered three times per week for eight weeks.

44. The method of claim 14, wherein said liposome consists essentially of neutral phospholipids.

46. The method of claim 24, wherein said liposome consists essentially of neutral phospholipids.

58. The composition of claim 86, wherein said first polynucleotide is an oligonucleotide having a length of between about 8 and about 50 bases.

59. The composition of claim 86, wherein the first polynucleotide is complementary to the translation initiation site of Bcl-2 mRNA.

60. The composition of claim 59, wherein the polynucleotide is an oligonucleotide comprising the sequence CAGCGTGCGCCATCCTTC (SEQ ID NO:1).

61. The composition of claim 86, comprising a liposome formed from the phospholipid.

62. The composition of claim 61, wherein the first polynucleotide is encapsulated in the liposome.

63. The composition of claim 86, wherein the phospholipid is a phosphatidylcholine, a phosphatidylglycerol, or a phosphatidylethanolamine.

64. The composition of claim 63, wherein the phospholipid is dioleoylphosphatidylcholine.

65. A composition comprising an expression construct that encodes a first antisense polynucleotide that hybridizes to a second, Bcl-2-encoding polynucleotide under intracellular conditions, wherein said construct is under the control of a promoter that is active in eukaryotic cells and associated with a neutral phospholipid, wherein said first polynucleotide comprises at least 8 nucleotides of the sequence CAGCGTGCGCCATCCTTC (SEQ ID NO:1), wherein said polynucleotide is complementary to the translation initiation site of Bcl-2, further comprising a charged phospholipid.

72. A composition comprising a neutral phospholipid associated with an expression construct that encodes an oligonucleotide of from about 8 to about 50 bases and which hybridizes to Bcl-2 mRNA under intracellular conditions, wherein the construct is under the control of a promoter that is active in eukaryotic cells, further comprising a charged phospholipid.

73. The composition of claim 86, wherein said first polynucleotide is a P-ethoxy oligonucleotide.

74. The composition of claim 61, wherein said liposome consists essentially of neutral and charged phospholipids.

75. The composition of claim 65, comprising a liposome formed from said neutral phospholipid.

76. The composition of claim 75, wherein said liposome consists essentially of neutral and charged phospholipids.

79. The composition of claim 72, comprising a liposome formed from the phospholipid.

80. The composition of claim 79, wherein said liposome consists essentially of neutral and charged phospholipids.

81. A composition comprising a first antisense polynucleotide that hybridizes to a second, Bcl-2-encoding polynucleotide under intracellular conditions and a primary phosphatide associated with said first polynucleotide, wherein said primary phosphatide is a neutral phospholipid, and wherein said first polynucleotide comprises at least 8 nucleotides of the sequence CAGCGTGCGCCATCCTTC (SEQ ID NO:1), and wherein said polynucleotide is complementary to the translation initiation site of Bcl-2, further comprising a charged phospholipid.

82. The composition of claim 81, comprising a liposome formed from the primary phosphatide.

83. The composition of claim 82, wherein said liposome consists essentially of neutral and charged phospholipids.

84. The composition of claim 81, wherein said first polynucleotide is a P-ethoxy oligonucleotide.

85. The composition of claim 86, wherein said at least 8 nucleotides are consecutive nucleotides.

86. A composition comprising a first antisense polynucleotide that hybridizes to a second, Bcl-2-encoding polynucleotide under intracellular conditions and a neutral phospholipid associated with said first polynucleotide, to form a Bcl-2 polynucleotide/neutral phospholipid association, wherein said first polynucleotide comprises at least 8 nucleotides of the sequence CAGCGTGCGCCATCCTTC (SEQ ID NO:1), wherein said polynucleotide is complementary to the translation initiation site of Bcl-2, said composition further comprising a charged phospholipid.

87. The composition of claim 86, wherein the charged phospholipid is a positively charged phospholipid.

88. A method of inhibiting proliferation of a Bcl-2-associated disease cell comprising obtaining a polynucleotide that hybridizes to Bcl-2 mRNA under intracellular conditions, mixing the first polynucleotide with a neutral phospholipid to form a composition comprising a polynucleotide/phospholipid association, and administering said composition to a human having a Bcl-2-associated disease to inhibit the proliferation of said disease cells, wherein said disease cells have a t(14;18) translocation, the composition further comprising a charged phospholipid.

89. The method of claim 88, wherein the charged phospholipid is a positively charged phospholipid.

91. The method of claim 19, wherein said first polynucleotide is a P-ethoxy oligonucleotide.

92. The method of claim 29, wherein said first oligonucleotide is a P-ethoxy oligonucleotide.